



## The Stimulatory Effect of *Lactobacillus plantarum* and *Bacillus lentus* on Yield and Nutrient Status of Faba Bean Grown under Newly Reclaimed Soil

Manal F. Mohamed<sup>1</sup>, Thalooth A.T.<sup>1</sup>, Salwa A. Orabi<sup>2</sup>, Foukia E. Mouafi<sup>3</sup> and Amal G. Ahmed<sup>1</sup>

<sup>1</sup>Field Crop Res. Dept., National Research Centre, El-Buhouth St., Dokki, Giza, Egypt.

<sup>2</sup>Botany Dept., National Research Centre, El-Buhouth St., Dokki, Giza, Egypt.

<sup>3</sup>Microbial Biotechnology Dept., National Research Centre, El-Buhouth St., Dokki, Giza, Egypt.

Received: 20 Sept. 2023

Accepted: 30 Oct. 2023

Published: 10 Nov. 2023

### ABSTRACT

At the current work ,two field experiment were carried out at the Agricultural Production Research Station , National Research Centre , Nubaria Province , Bahaira Governmate , Egypt , during the two successive winter seasons of 2020/2021 and 2021/2022 to study the promoting effect of foliar spraying of *Lactobaccillus plantarum* and *Bacillus lentus* on the yield and components of faba bean plants under newly reclaimed soils .The obtained results revealed that either *L. plantarum* or *bacillus lentus* treatment significantly enhanced all yield characters over untreated plants. Nitrogen, phosphorus and potassium contents of seeds also positively affected by *Lactobaccillus plantarum* and *Bacillus lentus* treatment but such effect was more pronounced under low concentration used. According to the obtained results it could be concluded that, the enhancing effect of the two microorganisms depends on the concentration of the used dose.

**Keywords:** Faba bean, *Lactobacillus plantarum*, *Bacillus lentus*, yield, newly reclaimed soil.

### 1. Introduction

Faba bean (*Vicia faba* L.), is the fourth most important pulse crop in the world (Sainte, 2011). It has a multipurpose use and is consumed as dry seeds, green vegetable, or as processed food. It is regarded as a critical legume crop used in human and animal nutrition. Faba bean (*Vicia faba* L.) is one of essential winter legume crops in Egypt due to its high nutritive value and high protein contents (20–30%) (Qahtan, *et al.*, 2021) also nutritive minerals, such as phosphorus, potassium, calcium, sulphur and iron (Matthews and Marcellos, 2003). Faba bean also is a major food and feed legume containing protein and 51–68% carbohydrate (Hendawey and Younes 2013) according to, the genotype. Moreover, Faba bean cultivation increases the sustainability of cropping systems by adding nitrogen (N) to the soil through symbiotic N<sub>2</sub> fixation which, improving soil health (Rose *et al.*, 2016).

The interaction between plants and microbes is an integral part of sustainable agriculture. Therefore, microbial-based agricultural practices and advancements could promote plant health and soil fertility There is an increasing demand on the productivity of crops, and the use of biofertilizers in the production that plays an important role as a supplement to improve the growth and yield of several agricultural plants. Plant growth-promoting rhizobacteria (PGPR) are able to facilitate plant nutrient acquisition. Several *Bacillus* species have been identified as plant growth-promoting bacteria since they suppress pathogens, otherwise promote plant growth. Improvements in plant health and productivity are mediated by secretion of compounds that promote the plant growth.

Lactic acid bacteria and other *Bacillus*-based bio fertilizers have been validated with established microbes in agriculture and the environment. Microbial-based biofertilizers increase crop yield and accelerate the mineral update of the plant root. LAB and *bacillus*-based biofertilizers showed a high crop yield and enhanced the organic matter degradation (Blais, 2006)

**Corresponding Author:** Manal F. Mohamed, Field Crop Res. Dept., National Research Centre, El-Buhouth St., Dokki, Giza, Egypt.

LABs have been reported to show biological control activity and plant growth-promoting activity in plants (Lutz *et al.*, 2012). The presence of LAB on plants definitely puts forth their benefits to plants one of which could be plant growth promotion. On other hand, LAB promote seed germination, increase soil fertility, aeration, and solubility, alleviate various abiotic stress, and neutralize toxic gasses Few studies elucidated the role of lactic acid bacteria (LAB) in the rhizosphere and their plant growth promoting properties. (Fhoula, *et al.*, 2013). Thus, the aim of this work is to study the effect of inoculation with *Lactobacillus plantarum* and *Bacillus lentus* on Faba bean growth and yield.

## 2. Material and Methods

At Production and Research Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt two field experiments were carried out on Faba bean during the growing seasons of 2020/2021 and 2021/2022 Faba bean was sprayed with different broth doses of *Lactobacillus plantarum* and *Bcillus lentus* to study the effect of these two microorganisms on growth and yield of broad bean. This experiment includes five treatments in three replicates with control (without foliar spraying), foliar spraying with 20% *Bacillus lentus*, foliar spraying with 40% *Bacillus lentus*, foliar spraying with *Lactobacillus plantarum* at 20% and foliar spraying with *Lactobacillus plantarum* at 40% concentration.

Faba bean (*Vicia faba* L.), variety Nobaria 1 was sown on 1<sup>st</sup> of December in both seasons in sandy soil. Bean cultivars were obtained from Food Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. All bean seeds used in the experiments for the two seasons were inoculated with the specific bacterial strain before sowing. Faba bean seeds were sown at rate of 20 kg seeds /fed. for all treatments. Calcium superphosphate (15.5 % P<sub>2</sub>O<sub>5</sub>) was applied during soil preparation at the rate of 100 kg/fed. Nitrogen in the form of ammonium nitrate (33% N) at the rate of 15 kg N/fed. was added as starter dose before the first irrigation. Potassium sulphate (48 % K<sub>2</sub>O) at the rate of 50 kg/fed. was applied to soil after 35 days from sowing. All the other recommended agricultural practices for faba bean production were applied at the proper time as recommended for this District by the Ministry of Agriculture. Plot size was 10.5 m. (3 m length x 3.5 m width) 1/400 fed. after one month of sowing the plants were sprayed with *Bacillus lentus* and *Lactobacillus plantarum* in concentration as indicated before (conc 1:20% & conc 2:40%). The experimental design was complete block design with three replicates.

Soil samples of experimental site were randomly collected before sowing to determine some physical and chemical properties of the soil for the two seasons according to Klute (1986) as shown in Table (1).

**Table 1:** Mechanical and chemical analyses of the experimental soil (2020/2021 and 2021/2022 seasons).

Mechanical analysis	2020/2021	2021/2022
Sand%	92.0	90.9
Silt	3.3	4.1
Clay	4.7	5.0
<b>Chemical analysis</b>		
CaCO <sub>3</sub>	1.3	1.5
Organic matter%	0.4	0.4
EC mmhos/cm <sup>2</sup>	0.3	0.3
pH	7.4	7.3
Soluble N	8.2	8.3
Available P(ppm)	3.0	3.6
Available K(ppm)	20.0	21.2

At harvesting time, plant height (cm), number of branches, plant weight(gm), pods weight (gm)and seed yield per plant(gm) and weight of 100 seeds were estimated. Straw yield, seed yield as well as biological yield (Kg/fed.) were determined for each plot.

Statistical analysis was performed according to Snedcor and Cochran (1990), treatment means were compared using least significant difference LSD at probability level of 5%.

### 3. Results and Discussion

#### 3.1. Effect of foliar application of *Lactobacillus plantarum* and *Bacillus lentus* on yield and yield attributes of Faba bean grown under newly reclaimed soil

Data presented in Table (2) indicate, that foliar application with *Lactobacillus plantarum* under both concentrations significantly increased most of yield characters as plant height; weight of plant; number of branches, Pod weight and seed weight/ plant as compared with untreated plants (control). These results are in agreement with those obtained by Kang *et al.*, (2015) and Giassi *et al.*, (2016). Also, these results coincide with results obtained by Nataliaia Limanska *et al.*, (2013) who reported that inoculation with *Lactobacillus plantarum* stimulated the main root growth and shoot growth. In this respect, Merlich *et al.*, (2017) also reported that *L. plantarum* had the stimulation effect on height of wheat seedlings. On other hand recently, Raman *et al.*, (2022) reported that lactic acid bacteria metabolites, promote plant growth; stimulate shoot and root growth.

The same Table also shows that inoculation with *Bacillus lentus* in both concentrations have more promoting effect on plant height; weight of plant; number of branches; Pod weight and seed weight/ plant, than *Lactobacillus plantarum*. Such effect may be due to *Bacillus lentus* effect on facilitating plant nutrient acquisition also, improving plant health and productivity (Wang *et al.*, 2019). In this concern, Lai *et al.*, (2023) reported that plant weight and plant length grew approximately by 1.5–1.9 times and 1.4–1.6 times, respectively of peanut plants by Bacillus application. So, inoculation with this microorganism may enhancing secretion Such promoting effect of this bacteria may resulted in secretion of compounds that promote the plant growth and stimulation of plant host.

Accordingly, Table (2) show that *Bacillus lentus* has enhancing effect on yield of faba bean, such effect reflected by high values of weight of 100 seeds, seed yield/ feddan, straw yield and biological yield per feddan. Such pronounced effect of Bacillus sp. on yield reported by other investigators (Bisen *et al.*, 2015; Mishra *et al.*, 2015). These results may be due to beneficial interactions of this microorganism in increasing crop nutrients; stimulation of plant growth producing phytohormones, 1-aminocyclopropane -1-carboxylate deaminase, siderophore production, and phosphate solubilization additionally, improving soil structure; bioaccumulation of inorganic compounds; and bioremediation of metal-contaminated soils (Singh *et al.*, 2016, 2017). On other hand, PanelShalini *et al.*, (2019) added that Bacillus sp. is one of the most extensively studied rhizobacteria that promotes plant growth and development.

**Table 2:** Effect of *Lactobacillus plantarum* and *Bacillus lentus* treatment on yield of faba bean under newly reclaimed soil

Treatment	Yield characters				
	Plant length cm	Plant weight gm	Branches No.	Pod weight /plant (gm)	Grain weight /plant (gm)
Control	51.00	30.12	10.04	47.67	24.50
Lacto (conc.1)	56.00	40.07	13.36	57.67	28.52
Lacto (conc.2)	51.89	39.50	13.17	77.33	30.04
Bacillus (conc.1)	63.44	38.26	12.75	89.00	35.08
Bacillus (conc.2)	48.22	35.06	11.69	60.67	30.87
LSD at 5%	8.99	13.22	0.80	19.88	0.41

**Table 2:** Continue

Treatment	Yield characters			
	100 grain weight gm	Grain yield Kg/ feddan	Straw yield Kg/ feddan	Biological yield Kg/ feddan
Control	75.90	1028.33	278.33	1306.67
Lacto (conc.1)	88.37	1197.67	344.67	1542.33
Lacto (conc.2)	75.92	1261.67	403.00	1664.67
Bacillus (conc.1)	80.49	1472.33	401.00	1873.33
Bacillus (conc.2)	85.03	1296.67	375.67	1672.33
LSD at 5%	6.38	84.60	221.25	211.17

The enhancing effect of *Lacto bacillus* on faba bean yield also reported by many investigators (Abhyankar, *et al.*, 2021 and Strafella *et al.*, 2021). also Strafella *et al.*, (2021) reported that the lactic

acid bacteria showed various characteristics which can be related as support to plant growth. They added that lactic acid bacteria inhabiting plant surfaces produce plant growth promoting hormones and hence can be used to promote the plant growth. On other hand Primavesi (2020), reported that the highest field bean yields (2087 kg ha-t) were obtained with Lactic acid bacteria treatment spray applied at (0.02%). It could be concluded that Lactic a.b. is the most expedient means of maximizing bean yields. Raman *et al.*, (2022) added that LAB and bacillus-based biofertilizers showed a high crop yield and enhanced the organic matter degradation.

Regarding the effect of doses rate foliar application of *Lactobacillus plantarum* and *Bacillus lentus* on yield and growth of faba bean, the data recorded in Table 2 indicate that using of higher concentration of Lactic acid bacteria, the higher yield of faba bean obtained. Similar results was obtained by Merlich *et al.*, (2017) who reported that, the influence of lactic acid bacteria depended both on the strains and concentrations of bacterial cells. Simliar results obtained by Primavesi (2020) On the other hand, the same table show that foliar application of low dose of Bacillus significantly increased yield focused by high yield of seeds, straw and biological yield per faddan.

### 3.2. Effect of foliar application of *Lactobacillus plantarum* and *Bacillus lentus* on the chemical constituents of the seed

Data presented in Table (3) clearly indicate that either *Lactobacillus plantarum* or *Bacillus lentus* treatment increased the chemical constituents of the seeds (N, P and K) as compared with untreated plants.

Similar results obtained by Wang *et al.*, (2019) who reported that most plant-growth-promoting microorganisms can promote plant growth and accelerate nutrient availability and uptake. They also added that microbial-based biofertilizers increase crop yield and accelerate the mineral update of the plant root. In this concern, Figueiredo *et al.* (2016) reported that PGPR Bacillus has the ability for nutrient supply (nitrogen, phosphorus, potassium and essential minerals).

The same table (3) also shows that, nitrogen concentration of the seeds is affected by the dose of *Lactobacillus plantarum* and *Bacillus lentus*. In general, using of the low dose increased nitrogen concentration in the seeds Such promoting effect of both Lactic acid bacteria and Bacillus sp. on nitrogen uptake supported by Figueiredo *et al.* (2016) and Cacace *et al.* (2020) who reported that some hypothetical views revealed that LAB fix atmospheric nitrogen.

Also Phosphorus content in the seeds positively affected by Lactic acid bacteria and Bacillus treatment as compared with control. However, the data shows that on contrast to nitrogen, phosphorus uptake enhanced by high level of either Lactic a.b. or Bacillus sp. treatment. The positive explanation regarding enhancing effect of both microorganism indicated by Figueiredo *et al.* (2016) and Coccozza and Ercolani (1997) who reported that LAB-based composting materials promote phosphorous and iron precipitates, such as Ca phosphates and iron oxides.

Potassium content of the faba bean seeds also responded positively to foliar application of either Lactic acid bacteria or *Bacillus lentus* treatment and such effect was more pronounced under high level of application. These results are in agreement with Figueiredo *et al.* (2016) and Wang *et al.* (2019).

**Table 3:** Effect of *Lactobacillus plantarum* and *Bacillus lentus* treatment on chemical constituents of faba bean seeds under newly reclaimed soil.

Treatment	N %	P %	K %
Control	2.77	0.14	0.96
Lacto (conc. 1)	3.11	0.15	0.98
Lacto (conc. 2)	2.83	0.16	1.02
Bacillus (conc. 1)	3.00	0.13	1.01
Bacillus (conc. 2)	2.88	0.16	1.03

### Conclusion

Sustainable agriculture has recently been more concerned with a sustainable food system; also farming is most important for global health. Microbial-based agricultural practices would help alleviate these concerns and supply sufficient food for the world population. Lactic acid bacteria; Bacillus sp. - based biofertilizers; antimicrobial on other hand growth-promoting compounds can replace inorganic

fertilizer. Thus, on the base of the obtained results it could be concluded that *Lactobacillus plantarum* and *Bacillus lentus* are a promising candidate for sustainable agriculture under newly reclaimed soil.

## References

- Abhyankar, P.S., A.B. Gunjal, B.P. Kapadnis, and S.V. Ambade, 2021. Potential of Lactic Acid Bacteria in Plant Growth Promotion. *Bhartiya Krishi Anusandhan Patrika*, 36(4): 326-329 DOI: 10.18805/BKAP374
- Bisen, K., C. Keswani, S. Mishra, A. Saxena, A. Rakshit, and H.B. Singh, 2015 Unrealized potential of seed biopriming for versatile agriculture. In: Rakshit A, Singh HB, Sen A (eds) *Nutrient use efficiency: from basics to advances*. Springer, New Delhi, 193–206.
- Blais, A., 2006. Lactic Acid and Bacillaceae Fertilizer and Method of Producing Same. Canadian Patent No. CA2598539A1.
- Cacace, C., C.G. Rizzello, G. Brunetti, M. Verni, and C. Coccozza, 2022. Reuse of Wasted Bread as Soil Amendment: Bioprocessing, Effects on Alkaline Soil and Escarole (*Cichorium endivia*) Production. *Foods*, 11: 189.
- Coccozza, C., and G.L. Ercolani, 1997. Siderophore production and associated characteristics in rhizosphere and non-rhizosphere fluorescent pseudomonads. *Ann. Microbiol.*, 47: 17–28
- Fhoula, I., A. Najjari, Y. Turki, S. Jaballah, A. Boudabous, and H. Ouzari, 2013. Diversity and antimicrobial properties of lactic acid bacteria isolated from rhizosphere of olive trees and desert truffles of Tunisia. *Biomed. Res. Int.*, 405708.
- Figueiredo, M.V.B., A. Bonifacio, A.C. Rodrigues, and F.F. Araujo, 2016 Plant growth-promoting rhizobacteria: key mechanisms of action. In: Choudhary DK, Varma A (eds) *Microbial-mediated induced systemic resistance in plants*. Springer Science + Business Media, Singapore, 23–37
- Giassi, V., C. Kiritani, and K.C. Kupper, 2016. Bacteria as growth-promoting agents for citrus rootstocks. *Microbiol. Res.*, 190: 46–54.
- Hendawey, M. and A. Younes, 2013. Biochemical Evaluation of Some Faba Bean Cultivars under Rainfed Conditions at El-Sheikh Zuwayid. *Annals of Agricultural Sciences*, 58: 183-193. <http://dx.doi.org/10.1016/j.aosas.2013.07.010>
- Kang, S.M., R. Radhakrishnan, Y.H. You, A.L. Khan, J.M. Park, S.M. Lee, I.J. Lee, 1995. Cucumber performance is improved by inoculation with plant growth-promoting microorganisms. *Acta Agric. Scand. B soil Plant Sci.* 2015, 65, 36–44. Kyusei Nature Farming, Paris, France, 154–158.
- Klute, A., 1986. *Methods of soil analysis*. 2nd ed. Part 1: physical and mineralogical methods. Part 2: chemical and microbiological properties. Madifon, Wesconsin, USA
- Lai, L.T., L.N. Ai Mi, and H.N. Hoai 2023. Root-associated bacteria *Bacillus albus* and *Bacillus proteolyticus* promote the growth of peanut seedlings and protect them from the aflatoxigenic *Aspergillus flavus* CDP2. *Biocatalysis and Agricultural Biotechnology* Volume 47, January 2023, 102582.
- Lutz, M.P., V. Michel, C. Martinez, and C. Camps, 2012. Lactic acid bacteria as biocontrol agents of soil-borne pathogens biological control of fungal and bacterial plant pathogens. *Biol. Control. Fungal Bact. Plant Pathog.* 78: 285–288
- Matthews, P. and H. Marcellos, 2003. Faba Bean. *Agfact P4.2.7.*, Second Edition 2003, Division of Plant Industries, New South Wales Agriculture, Australia, 1-12.
- Merlich A.G., N.V. Limanska, I.D. Zhunko, and D.O. Babenko, 2017. Effect of *Lactobacillus plantarum* and *Bacillus Atrophalus* on germination of wheat seedlings growth. *Microbiology & Biotechnology*. 1(37):96582.
- Mishra, S., A. Singh, C. Keswani, A. Saxena, B.K. Sarma, and H.B. Singh, 2015. Harnessing plant-microbe interactions for enhanced protection against phytopathogens. In: Arora NK (ed) *Plant microbe Symbiosis—applied facets*. Springer, New Delhi, 111–125.
- Nataliia, L., I. Tetiana, B. Olena and K. Kateryna, 2013. Effect of *Lactobacillus plantarum* on germination and growth of tomato seedlings. *Physiologiae Plantarum*, 35(5).
- PanelShalini, T., P. Vivek, and L. Charu, 2019. *Microbial Biotechnology in Agro-Environmental Sustainability*. Bio technology and bio Engineering, 43-55.
- Primavesi, A.M., 2020 Effect of *Lactobacillus* Inoculants, Organic Amendments and Mineral Elements on Yield of Onion and Field Bean. *Fazenda Ecológica Itai, SP, Brazil*

- Qahtan, A.A., A. Al-Atar, E.M. Abdel-Salam, M.A. El-Sheikh, A.-R.Z. Gaafar, and M. Faisal, 2021. Genetic diversity and structure analysis of a worldwide collection of faba bean (*Vicia faba*) genotypes using ISSR markers. *Int. J. Agric. Biol.*, 25: 683–691.
- Raman, J., J.-S. Kim, K.R. Choi, H. Eun, D. Yang, Y.J. Ko, and S.J. Kim, 2022. Application of Lactic Acid Bacteria (LAB) in Sustainable Agriculture: Advantages and Limitations. *Int. J. Mol. Sci.*, 7784.
- Rose, T.J., C.C. Julia, M. Shepherd, M.T. Rose, and L. Van Zwieten, 2016 Faba bean is less susceptible to fertiliser N impacts on biological N<sub>2</sub> fixation than chickpea in monoculture and intercropping systems. *Biology and Fertility of Soils*, 52: 271–276.
- Sainte, M., 2011. The magazine of the European Association for Grain Legume Research. Issue No. 56 Model Legume Congress, France, 15-19 .
- Singh, H.B., B.K. Sarma, C. Keswani, eds, 2016. Agriculturally important microorganisms: commercialization and regulatory requirements in Asia. Springer, Singapore
- Singh, H.B., B.K. Sarma, C. Keswani, eds, 2017. Advances in PGPR. CABI, Wallingford.
- Snedecor, G.W. and W.G. Cochran, 1990. "Statistical Methods" 8th ed., Iowa at. Univ. Press, Ames, Iowa, USA
- Strafella, S., D.J. Simpson, M.Y. Khanghahi, M.D. Angelis, M. Gänzle, F. Minervini, and C. Crecchio, 2021 Comparative genomics and in vitro plant growth promotion and biocontrol traits of lactic acid bacteria from the wheat rhizosphere. *Microorganisms*. 9(1): 78.
- Wang, Y., L. Bi, Y. Liao, D. Lu, H. Zhang, X. Liao, J.B. Liang, and Y. Wu, 2019. Influence and characteristics of *Bacillus stearothermophilus* in ammonia reduction during layer manure composting. *Ecotoxicol. Environ. Saf.*, 180: 80–87.